

SN 09/856,907  
Atty Docket No. DSP/HB/07.01/US

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### AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### LISTING OF CLAIMS:

1. (Currently Amended) A method of preparing a nucleic acid library, said method comprising introducing at least two members of an initial population of nucleic acid molecules into at least one cell, wherein members of the initial population of nucleic acid molecules comprise two sequence segments,

(a) wherein all first sequence segments are identical and include at least one origin of replication; a nucleic acid vector sequence that is identical for each member of the initial population of nucleic acid molecules and that includes an origin of replication; and,

(b) wherein the second sequence segment comprises a library of diverse expression sequences, wherein each of the diverse expression sequences in the library is different and comprises a nucleic acid sequence that varies between members of the initial population of nucleic acid molecules and comprises at least one substrate for recombination,

such that recombination of the substrate occurs between at least two members of the initial population of nucleic acid molecules, thereby producing a population of nucleic acid molecules comprising recombined nucleic acid members.

2. (Original) The method of claim 1, wherein said recombination is performed by a recombination mechanism endogenous to said cell.

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3. (Withdrawn) The method of claim 1, wherein said recombination is mediated by an exogenous recombinase.
4. (Original) The method of claim 1, wherein said recombination is mediated by an endogenous recombinase.
5. (Original) The method of claim 1, wherein said recombination is at a site preselected for recombination.
6. (Original) The method of claim 5, wherein the site preselected for recombination is a recombinase recognition site and the recombination is mediated by a recombinase expressed by said cell.
7. (Original) The method of claim 1, wherein said recombination is mediated by a recombinase selected from the group consisting of a member of the *hin* family of recombinases, a member of the *lambda* integrase family, an *flp* recombinase, a resolvase, a transposon, and a *Cre* recombinase.
8. (Original) The method of claim 7, wherein said recombinase is selected from the group consisting of *Cre*, *hin*, *gin*, *pin*, *cin*, and *flp*.
9. (Original) The method of claim 5, wherein said site preselected for recombination is a *loxP* site.
10. (Previously Amended) The method of claim 1, wherein said substrate for recombination comprises:
  - (i) a first site recombinase recognition site; and
  - (ii) a second recombinase recognition site different from the first recombinase recognition site.

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11. (Original) The method of claim 10, wherein recombination results in the exchange, between two members of said nucleic acid population, of the nucleic acid flanked by the first and second recombinase recognition sites.

12. (Original) The method of claim 10, wherein the first recombinase recognition site is a loxP site and the second recombinase recognition site is loxP mutant site.

13. (Original) The method of claim 12, wherein the loxP mutant site is loxP 511.

14. (Original) The method of claim 1, wherein said cell is selected from the group consisting of a bacterial cell, a yeast cell, an insect cell, and a mammalian cell.

15. (Original) The method of claim 14, wherein said bacterial cell is an *Escherichia coli* cell.

16. (Original) The method of claim 1, wherein said members of a population of nucleic acid molecules are introduced into the cell by transfection.

17. (Original) The method of claim 1, wherein said population of nucleic acid molecules comprises at least 10 different members.

18. (Original) The method of claim 1, wherein said members of a population of nucleic acid molecules are contained within infectious particles and are introduced into the cells via infection with said infectious particles.

19. (Original) The method of claim 18, wherein said infectious particles are phage.

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20. (Original) The method of claim 19, wherein said infectious particles are filamentous phage.
21. (Original) The method of claim 20, wherein the infectious particles are filamentous phage of the Ff family.
22. (Original) The method of claim 18, wherein said infectious particles are phagemids containing phagemidic DNA.
23. (Original) The method of claim 18, wherein said infectious particles are phagemids derived from filamentous phage of the Ff family.
24. (Original) The method of claim 1, wherein said method further comprises:  
transfecting or infecting one or more cells with members of said population of recombinant nucleic acid members such that said cells are infected at a multiplicity of infection (moi) of less than about 1.
25. (Original) The method of claim 24, wherein said further method comprises the packaging of members of said nucleic acid library in replicable genetic display packages such that a protein on the surface of the replicable display package is encoded by a nucleic acid packaged within the display package that is a nucleic acid sequence that varies between members of the nucleic acid library.
26. (Original) The method of claim 1, wherein the variable nucleic acid sequence comprising the substrate for recombination comprises an expression cassette.
27. (Original) The method of claim 26, wherein said expression cassette comprises nucleic acid sequences encoding one or more polypeptides.

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28. (Original) The method of claim 26, wherein said expression cassette comprises nucleic acid sequences encoding one or more polypeptides and the nucleic acid encoding at least one of said polypeptides is flanked by pair of recombinase recognition sites.

29. (Original) The method of claim 27, wherein said polypeptides are expressed on the surface of a phage, a phagemid, or a bacterium.

30. (Original) The method of claim 27, wherein said variable sequence includes nucleic acid encoding a first polypeptide chain and a second polypeptide chain from a specific binding pair member such that following recombination said variable sequence encodes binding proteins that are not present in the initial population of nucleic acids.

31. (Original) The method of claim 30, wherein said first and said second polypeptide are antibody polypeptides.

32. (Original) The method of claim 31, wherein said first and second polypeptide are selected from the group consisting of a V<sub>H</sub> region, a V<sub>L</sub> region, a V<sub>H</sub> CDR1, a V<sub>H</sub> CDR2, a V<sub>H</sub> CDR3, a V<sub>L</sub> CDR1, a V<sub>L</sub> CDR2, a V<sub>L</sub> CDR3, a V<sub>H</sub> joined to a C<sub>H</sub>1, and a V<sub>L</sub> joined to a C<sub>L</sub>.

33. (Original) The method of claim 32, wherein the first polypeptide is a V<sub>H</sub> region and the second polypeptide is a V<sub>L</sub> region.

34. (Previously Amended) The method of claim 30, wherein

(i) a pair of recombinase recognition sites flank the nucleic acid encoding a first polypeptide; and,

(ii) said pair of recombinase recognition sites comprise a first recombinase recognition site and a different second recombinase recognition site.

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35. (Original) The method of claim 34, wherein the first recombinase recognition site is a LoxP site and the second recombinase recognition site is a LoxP 511 site.

36. (Original) The method of claim 30, wherein the first polypeptide is flanked by a pair of recombinase recognition sites and the recognition sites are different from each other.

37. (Previously Amended) The method of claim 30, wherein

(i) the first polypeptide and the second polypeptide are each flanked by a pair of recombinase recognition sites; and,

(ii) the recognition sites within each pair are different from each other.

38. (Original) The method of claim 36, wherein said loxP sites are selected from the group consisting of loxP, loxP 511, and fas loxP.

39. (Original) The method of claim 29, wherein the members of said library encode a single-chain antibody.

40. (Original) The method of claim 39, in which said antibody fragments are scFv.

41. (Original) The method of claim 29, wherein the members of said library encode a moiety selected from the group consisting of a Fab, an Fv, a diabody, a V<sub>H</sub> dimer, and a V<sub>L</sub> dimer.

42. (Original) The method of claim 29, wherein the members of said library encode an antibody in which the antibody V regions are linked by a polypeptide linker comprising a recombinase recognition site.

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43. (Previously Amended) The method of claim 42, wherein said recombinase recognition site is selected from the group consisting of loxP, a loxP mutant, a recognition site for a hin family recombinase, a recognition site for a lambda integrase, a recognition site for an flp recombinase, a recognition site for a resolvase, and a recognition site for a transposon.

44. (Currently Amended) A method according to claim 1, wherein the second sequence segment~~variable portion of the nucleic acid~~ further comprises a selectable marker whereby said selectable marker must be recombined with a second selectable marker to become active.

45. (Cancelled)

46. (Original) The method of claim 44 wherein said selectable marker is inactive without recombination because it is an incomplete selectable maker.

47. (Original) The method of claim 44 wherein said selectable marker is located such that it is linked to the recombination substrate and co-transferred with a gene of interest in said recombination substrate.

48. (Original) A nucleic acid library made according to the method of claim 1.

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1. (Currently Amended) A method of preparing a nucleic acid library, said method comprising introducing at least two members of an initial population of nucleic acid molecules into at least one cell, wherein members of the initial population of nucleic acid molecules comprise two sequence segments,

(a) wherein all first sequence segments are identical and include at least one origin of replication; and,

(b) wherein the second sequence segment comprises a library of diverse expression sequences, wherein each of the diverse expression sequences in the library is different and comprise at least one substrate for recombination,

such that recombination of the substrate occurs between at least two members of the initial population of nucleic acid molecules, thereby producing a population of nucleic acid molecules comprising recombined nucleic acid members.

49. (Withdrawn – Currently Amended) A nucleic acid library comprising a population of nucleic acid molecules comprising two or more individual nucleic acids each of which consists of two sequence segments, wherein all first sequence segments are identical and include at least one origin of replication and at least two recombinase recognition sites, and wherein the second sequence segment comprises a library of diverse expression sequences, wherein each of the diverse expression sequences in the library is different and comprises at least one substrate for recombination~~nucleic acid sequence that is identical for each molecule and that includes an origin of replication and at least two recombinase recognition sites; and a nucleic acid sequence that varies between members of said population wherein said nucleic acid sequence that varies comprises a substrate for recombination, and wherein every member of said library has the same origin of replication.~~



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50. (Withdrawn) The nucleic acid library of claim 49, wherein said library comprises at least 10 different members in a single cell.
51. (Withdrawn) The nucleic acid library of claim 49, wherein said library comprises at least 100 different members in a single cell.
52. (Withdrawn) The nucleic acid library of claim 49, wherein said recombinase recognition sites comprise sites recognized by a recombinase selected from the group consisting of a member of the *hin* family of recombinases, a member of the *lambda* integrase family, an *flp* recombinase, a resolvase, a transposon, and a *Cre* recombinase.
53. (Withdrawn) The nucleic acid library of claim 52, wherein said recombinase is selected from the group consisting of *Cre*, *hin*, *gin*, *pin*, *cin*, and *flp*.
54. (Withdrawn) The nucleic acid library of claim 49, wherein said recombinase recognition site is a *LoxP* site or a mutant *LoxP* site.
55. (Withdrawn) The nucleic acid library of claim 49, wherein said members of a population of nucleic acid molecules are contained within infectious particles.
56. (Withdrawn) The nucleic acid library of claim 55, wherein said infectious particles are phage.
57. (Withdrawn) The nucleic acid library of claim 55, wherein said infectious particles are filamentous phage.
58. (Withdrawn) The nucleic acid library of claim 55, wherein said infectious particles are filamentous phages of the *Ff* family.
59. (Withdrawn) The nucleic acid library of claim 55, wherein said infectious particles are phagemid containing phagemidic DNA.
60. (Withdrawn) The nucleic acid library of claim 55, wherein said infectious particles are phagemids derived from filamentous phage of the *Ff* family.
61. (Withdrawn – Currently Amended) The nucleic acid library of claim 49, wherein the second sequence segment~~variable-nucleic-acid-sequence~~ comprising the substrate for recombination comprises and expression cassette.

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62. (Withdrawn) The nucleic acid library of claim 61, wherein said expression cassette comprises nucleic acid sequences encoding one or more polypeptides.

63. (Withdrawn) The nucleic acid library of claim 62, wherein said polypeptides are expressed on the surface of a phage or phagemid.

64. (Withdrawn – Currently Amended) The nucleic acid library of claim 49, wherein said second sequence segment~~variable sequence~~ includes nucleic acid encoding a first polypeptide chain and a second polypeptide chain from a specific binding pair member such that following recombination binding nucleic acids encoding binding proteins are produced that are not present in the initial population of nucleic acids.

65. (Withdrawn) The nucleic acid library of claim 64, wherein said first and said second polypeptide are antibody polypeptides.

66. (Withdrawn) The nucleic acid library of claim 65, wherein said first and second polypeptide are selected from the group consisting of a V<sub>H</sub> region, a V<sub>L</sub> region, a V<sub>H</sub> CDR1, a V<sub>H</sub> CDR2, a V<sub>H</sub> CDR<sub>3</sub>, a V<sub>L</sub> CDR1, a V<sub>L</sub> CDR<sub>2</sub>, a V<sub>L</sub> CDR<sub>3</sub>, a V<sub>H</sub> joined to a C<sub>H</sub>1, and a V<sub>L</sub> joined to a C<sub>L</sub>.

67. (Withdrawn) The nucleic acid library of claim 65, wherein the first polypeptide is a V<sub>H</sub> region and second polypeptide is a V<sub>L</sub> region.

68. (Withdrawn) The nucleic acid library of claim 64, wherein a pair of recombinase recognition sites flank the nucleic acid encoding a first polypeptide and pair of recombinase recognition sites comprise a first recombinase recognition site and a different second recombinase recognition site.

69. (Withdrawn) The nucleic acid library of claim 68, wherein the first recombinase recognition site is a LoxP site and the second recombinase recognition site is LoxP 511 site.

70. (Withdrawn) The nucleic acid library of claims 64, wherein the members of said library encode a single-chain antibody.

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71. (Withdrawn) The nucleic acid library of claims 64, wherein the first and the second polypeptide are expressed on the surface of a phage or bacterium.
72. (Withdrawn) The nucleic acid library of claim 70, in which said polypeptides are scFv, in which  $V_H$  and  $V_L$  are joined by a polypeptide linker encoded by a nucleic acid comprising a loxP, a loxP mutant, a recognition site for a *hin* family recombinase, a recognition site for a lambda integrase, recognition site for an *flp* recombinase, a recognition site for a resolvase, and a recognition site for a transposon.
73. (Withdrawn) The nucleic acid library of claims 49, wherein said nucleic acids express rgdp polypeptides.
74. (Withdrawn) A method of preparing a polypeptide said method comprising:
- a) providing a nucleic acid library of claims 48;
  - b) selecting one or more members of said library; and
  - c) expressing the nucleic acids of the one or more selected members.
75. (Withdrawn) The method of claim 74, wherein said selecting comprises:
- i) expressing proteins encoded by the members of said nucleic acid library; and
  - ii) screening the expressed proteins for one or more properties selected from the group consisting of specific binding to one or more preselected targets, a minimum binding avidity for one or more preselected targets, a maximum binding avidity for one or more preselected targets, thermostability at a particular preselected temperature, a predefined catalytic activity, a predefined enzymatic activity under selected conditions, a predefined biological activity; and
  - iii) selecting the library members that meet the screening criteria.
76. (Withdrawn) The method of claim 75, wherein said screening comprises screening for specific binding to a preselected target.
77. (Withdrawn) The method of claim 75, wherein the expressed proteins comprise single chain antibodies.

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78. (Withdrawn) The method of claim 75, wherein the polypeptides are expressed on the surface of cells infected or transfected with the members of the nucleic acid library.

79. (Withdrawn) The method of claim 75, wherein the polypeptides are expressed on the surface of replicable genetic display packages (rgdp).

80. (Withdrawn) The method of claim 75 where said members selected in step (iii) are used to enrich or generate a library according to the method of claim 1.

81. (Withdrawn) A procedure according to claim 79, in which said replicable genetic display package (rgdp) is a phagemid expressing one or more polypeptides bound to surface proteins.

82. (Withdrawn) A polypeptide encoded by a member of a nucleic acid library of claims 48.

83. (Withdrawn) A host cell comprising a nucleic acid library according to claims 48.

84. (Withdrawn) The host cell of claim 83, wherein said cell is selected from the group consisting of a bacterial cell, a plant cell, a mammalian cell, an insect cell, and a yeast cell.

85. (Withdrawn) A vector encoding a single chain antibody, where a nucleic acid encoding a fragment of said antibody is flanked by a pair or recombinase recognition sites where said recombinase recognition sites are different such that the nucleic acid encoding the fragment of the antibody can be exchanged between different plasmids of the same type via the action of a recombinase.

86. (Withdrawn) The vector of claim 85, wherein antibody fragment is selected from the group consisting of a  $V_H$  region, a  $V_L$  region, a  $V_H$  CDR1, a  $V_H$  CDR<sub>2</sub>, a  $V_H$  CDR<sub>3</sub>, a  $V_L$  CDR1, a  $V_L$  CDR<sub>2</sub>, a  $V_L$  CDR<sub>3</sub>, a  $V_H$  joined to a  $C_H1$ , and a  $V_L$  joined to a  $C_L$ .

87. (Withdrawn) The vector of claim 85, wherein said recombinase recognition sites are selected from the group consisting of loxP, a loxP mutant, a recognition site for a  $\text{hin}$  family recombinase, a recognition site for a lambda integrase,

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recognition site for an  $\phi$  recombinase, a recognition site for a resolvase, and a recognition site for a transposon.

88. (Withdrawn) The vector of claim 85, wherein said vector encodes an antibody comprising at least two antibody regions linked by a polypeptide linker comprising a recombinase recognition site.

89. (Withdrawn) The vector of claim 88, wherein said vector encodes an antibody in which the  $V_H$  and  $V_L$  regions are linked by a polypeptide linker comprising a recombinase recognition site.

90. (Withdrawn) The vector of claim 89, wherein said vector is pDAN5.

91. (Withdrawn) A kit comprising a container containing a vector of claims 85.

92. (Withdrawn) A kit comprising a container containing members of the nucleic acid library of claim 49.